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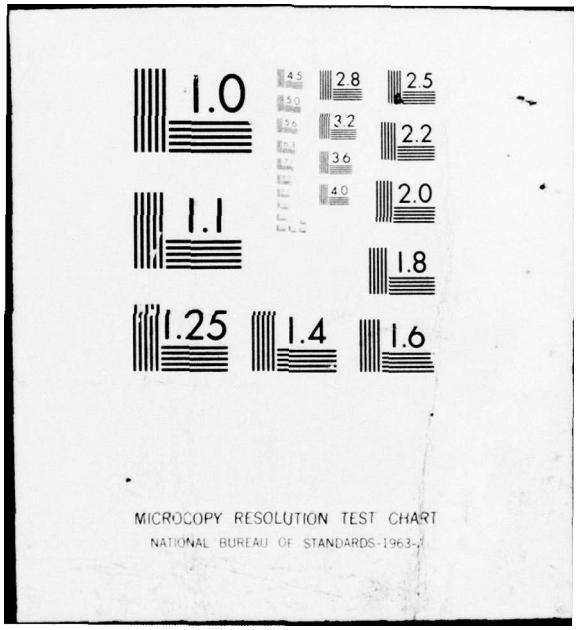
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IMMUNOLOGIC INTERRELATIONSHIPS OF COLIFORM HEAT-LABILE AND HEAT-STABLE ENTEROTOXINS

Annual Report

Frederick A. Klipstein, M.D.

February 15, 1978

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The goal of these studies is the development of an immunization program to prevent diarrheal disease due to intestinal contamination by toxigenic strains of coliform bacteria. Immunologic cross reactivity between cholera toxin and *E. coli* heat-labile (LT) toxin is well recognized. We showed that this immunologic interrelationship extends to the LT toxins of *Klebsiella pneumoniae* and *Enterobacter cloacae* and, in some instances, to the low molecular weight heat-stable (ST) toxins elaborated by these coliform bacteria.

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FORWARD AND ABSTRACT

This annual report describes research investigations conducted during the period between June 1, 1977 and February 15, 1978. Work completed during this period has either been: (a) published, (b) submitted for publication, or (c) put in preliminary draft form in preparation for submission for publication. This material is listed in this report under PUBLICATIONS AND MANUSCRIPTS and either a reprint or the manuscript form of each paper is enclosed. Since our work has been described in detail and the results discussed at length in this form, this annual report will confine itself to presenting a summary of what has been accomplished.

The goals of our research during the first year of support were: (a) to determine whether an immunologic interrelationship exists between the LT and ST enterotoxin forms of different species of coliform bacteria, and (b) to develop an animal model of acute and chronic intestinal contamination by coliform bacteria with specific toxigenic or nontoxigenic properties. These goals have been accomplished.

IMMUNOLOGICAL INTERRELATIONSHIPS BETWEEN CHOLERA TOXIN AND THE HEAT-LABILE AND HEAT-STABLE ENTEROTOXINS OF COLIFORM BACTERIA

Cholera toxin (CT) and the heat-labile (LT) toxin of Escherichia coli are known to share antigenic properties. This study examined the immunological relationship of CT and the LT and heat-stable (ST) toxins of E. coli, Klebsiella pneumoniae, and Enterobacter cloacae. The neutralizing capacity of equine CT antiserum and of antiserum raised in rabbits to the LT toxin of the three species of coliform bacteria was evaluated by determining their capacity to inhibit the action of purified CT and semipurified ultrafiltration preparations of the coliform LT and ST toxins in inducing water secretion as assayed by the in vivo marker perfusion technique in the rat jejunum. One milliliter of antiserum to CT and to E. coli and Klebsiella LT completely neutralized the secretory action of each of these three toxins; effective serial dilutions of CT antiserum extended to 1 to 4, whereas those of the antisera to LT were limited to 1 to 2 in most instances. One milliliter of antiserum to E. cloacae LT partially neutralized each of the three coliform LT toxins; serial dilutions were inactive. Antiserum to E. cloacae LT did not neutralize CT. Antiserum to CT and to each of the three coliform LT toxins also had a weak neutralizing effect on the ST toxins of E. coli and Klebsiella, but they did not affect E. cloacae ST. Adsorption of the antiserum to CT and to each of the three LT toxins by incubation with a heat-inactivated preparation of either the homologous or a heterologous LT toxin completely abolished the neutralizing capacity of the antisera towards both LT and ST. These observations indicate that the immunological interrelationship of CT and E. coli LT extends to the LT toxins of Klebsiella and E. cloacae and, further, that these immunological properties are shared to a lesser extent by the ST toxins of E. coli and Klebsiella.

IMMUNOLOGIC RELATIONSHIP OF DIFFERENT PREPARATIONS OF COLIFORM ENTEROTOXINS

Antisera raised in rabbits to LT preparations prepared either from whole cell lysates (LT-WCL) or broth filtrates (LT-BF) and to a broth filtrate ST preparation from nontoxigenic and toxigenic strains of Escherichia coli and Klebsiella pneumoniae were examined for their ability to neutralize the secretory effect of these toxins in the rat jejunum using in vivo marker perfusion

technique. Antisera to LT-WCL from nontoxigenic strains and antisera to the ST preparations from toxigenic strains were inactive. Antisera to both types of LT preparation from both bacterial species neutralized, with three exceptions, all of the homologous and heterologous LT and ST toxin preparations. When tested against material derived from whole cell lysates that had been separated on the basis of molecular weight by sequential ultrafiltration, antisera to the LT preparations consistently neutralized a heat-labile fraction with a defined molecular weight of between 30,000 and 100,000, but failed to neutralize a heat-stable fraction with a molecular weight of 500 to 30,000. These observations a) indicate that the immunologic interrelationship of E. coli and Klebsiella LT and ST toxins extends to antisera raised against LT prepared by different methods, b) confirm the lack of antigenicity of ST, and c) suggest an immunologic heterogeneity among different ST preparations based on their response to antisera to LT.

INTESTINAL STRUCTURE AND FUNCTION IN GERMFREE RATS CHRONICALLY COLONIZED BY ENTEROTOXIGENIC COLIFORM BACTERIA

Due to lack of a suitable model, the effect of either acute or chronic intestinal colonization by enterotoxigenic strains of coliform bacteria on intestinal transport has not been ascertained hithertofore. In the present study, groups of germfree rats were monocontaminated with either enterotoxigenic or nontoxigenic strains of Escherichia coli or Klebsiella pneumoniae and intestinal bacteriology, transport and structure were evaluated at weekly intervals for up to 5 weeks thereafter. Intestinal colonization occurred promptly; colony counts remained unchanged and the toxigenic strains continued to produce potent enterotoxins throughout the study period. Intestinal transport was assessed by the in vivo marker perfusion technique. Values in the jejunum and ileum of rats colonized by nontoxigenic strains were similar to those in conventional animals. Water and sodium transport was normal in the jejunum of animals contaminated by toxigenic E. coli during the first two weeks and in secretion thereafter; transport in the ileum was reduced after one week and in secretion thereafter. Water and sodium transport were either significantly reduced or in secretion throughout the study period in the jejunum of all rats contaminated with toxigenic K. pneumoniae. The absorption of xylose remained normal in all groups. Intestinal morphology was similar to that of conventional animals in most instances; no structural abnormalities that could specifically be attributed to the toxigenic strains were observed. These observations establish the fact that toxigenic strains of K. pneumoniae share with E. coli the capacity to alter water transport during intestinal colonization and indicate that chronic colonization by toxigenic strains of these species is associated with persistent abnormalities of intestinal transport.

REVERSAL OF JEJUNAL WATER SECRETION BY GLUCOSE IN RATS EXPOSED TO COLIFORM ENTEROTOXINS

Glucose absorption and glucose-facilitated water transport were assessed in rats exposed to semipurified preparations of the heat-labile (LT) and heat-stable (ST) enterotoxins of Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae by in vivo jejunal perfusion of these toxins alone and with varying amounts of glucose. Progressive increases in the glucose concen-

tration of from 12 to 56 mM resulted in incremental rises in water absorption, with Δ values that were equal to or exceeded that of the balanced electrolyte solution alone, in perfusates containing each of these toxins. Water secretion was reversed to absorption by the addition of 12 mM glucose when secretion was mild but 24 mM was required to achieve this in the presence of severe secretion. In no instance did absolute values for water transport return to normal. Glucose absorption was severely reduced from 24 and 56 mM solutions exposed to the ST toxins. It was marginally reduced by some of the LT toxins but this effect appeared to be due not to the toxin itself but rather to an unidentified factor that was also found present in the LT, but not the ST, preparations from nontoxigenic strains. These observations indicate that glucose-facilitated water transport remains intact in intestinal tissue exposed to various coliform enterotoxins and that this occurs despite the presence of impaired glucose absorption in some instances.

SUMMARY

These investigations provide evidence that an immunologic interrelationship exists between the LT and ST toxin forms of several species of coliform bacteria. Such a relation has previously been well documented to exist between E. coli LT and cholera toxin, and active immunization by cholera toxoid has been shown to protect partially against the action of E. coli LT in rats by Pierce. These observations suggest that active immunization using the LT toxin from one species of coliform bacteria may arouse protection against the secretory of this and the ST toxin form of multiple species of toxigenic coliform bacteria. We have shown in a preliminary study that active immunization against E. coli LT can be achieved in the rat. The next logical step is to determine whether active immunization is protective during acute and chronic intestinal contamination by toxigenic strains of different species of coliform bacteria. For this purpose, we have developed an animal model, the germfree rat, in which the pathophysiologic events during such colonization can be evaluated. We propose now to determine the protective effect of active immunization in this animal model.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

PUBLICATIONS AND MANUSCRIPTS

1. Klipstein FA, Engert RF: Immunological interrelationships between cholera toxin and the heat-labile and heat-stable enterotoxins of coliform bacteria. Infect Immun 18:110-117, 1977

2. Klipstein FA, Goetsch CA, Short HB, Engert RF, Schenk EA: Effect on small intestinal function and structure of chronic colonization by enterotoxigenic coliform bacteria in germfree rats. (Abstract) Gastroenterology 72:1081, 1977

3. Klipstein FA, Goetsch CA, Engert RF, Short HB, Schenk EA: Intestinal structure and function in germfree rats chronically colonized by enterotoxigenic coliform bacteria. Submitted for publication

4. Klipstein FA, Engert RF: Reversal of jejunal water secretion by glucose in rats exposed to coliform enterotoxins. Gastroenterology In press

5. Klipstein FA, Engert RF: Immunologic relationship of different preparations of coliform enterotoxins. Submitted for publication.

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